

Immunotherapy of Guinea Pigs with Dermal and Visceral Tumor Implants: Comparison of Living and Nonliving BCG

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Emulsified cell walls of *Mycobacterium bovis* (BCG) were immunotherapeutically at least as active as living BCG in prolonging survival of guinea pigs with established dermal tumors and microscopic lymph node and visceral metastases.

Living *Mycobacterium bovis*, strain BCG, has been administered intradermally (i.d.) (5, 6, 8, 9) or systemically (1, 2, 4) to cancer patients in attempts to eradicate localized and/or disseminated tumors remaining or recurring after other forms of treatment. One possible undesirable effect of administering living BCG is systemic BCG infection. Nonviable, but immunotherapeutically active, mycobacterial preparations might be useful clinically. Living BCG has been used to treat guinea pigs with established dermal tumors and artificial hematogenous metastases initiated by intravenous injection of tumor cells (3). The purpose of this study was to compare the efficacy of BCG cell walls (CW) (11) with living BCG in the treatment of guinea pigs with established dermal tumors and artificial visceral metastases.

All experiments were done with tumor line 10, an ascitic variant derived from a hepatocarcinoma induced by diethylnitrosamine in a strain 2 guinea pig. Inoculation of 10^6 tumor cells i.d. resulted in progressive i.d. tumor growth, and by 1 week tumor cells were present in the draining axillary lymph nodes; guinea pigs usually died 2 to 3 months later (7). Adult male syngeneic guinea pigs, Sewall-Wright strain 2, each received an i.d. injection of 10^6 line 10 tumor cells. Six days later, 5×10^4 or 5×10^5 tumor cells were injected intravenously. The next day (7 days after i.d. injection of tumor cells) animals in one group each received an intratumoral injection of 0.4 ml of viable BCG (Phipps substrain, TMC 1029, Trudeau Mycobacterial Collection, Saranac Lake, N.Y.) at 10^9 colony-forming units/ml. Animals in a second group each received an intratumoral injection of 0.85 mg of BCG CW in 0.4 ml of mineral oil (2%)-in-water emulsion prepared by the grinding method described by Yarkoni et al. (10). Statistical significance of differences in cure rates or in survival

times among treated and untreated animals was evaluated by the Wilcoxon nonparametric rank test.

In two separate experiments artificial hematogenous metastasis was induced with 5×10^4 tumor cells 6 days after the i.d. injection of 10^6 tumor cells. In the first experiment administration of living BCG intratumorally 1 day after intravenous injection of tumor cells rendered 3 of 14 animals free of gross metastasis at 167 days (Fig. 1); 8 of 15 animals treated with CW were tumor-free. No untreated control animals survived more than 70 days after i.d. tumor inoculation. In the second experiment living BCG and CW were equally effective; 2 of 14 animals were tumor-free at 180 days, and the median survival times were similar: 97 and 109 days, respectively. No untreated animals were alive at 61 days. The difference in prolongation of survival between the treated animals and the untreated controls was significant ($P < 0.001$). In a third experiment, 5×10^5 rather than 5×10^4 tumor cells were injected intravenously. As in the second experiment, CW was as effective as living BCG: 2 out of 14 and 1 out of 14 animals, respectively, were tumor-free at 180 days. No untreated animals survived more than 64 days. The prolongation of survival of animals treated with living BCG (median survival time, 73 days) or CW (median survival time, 89 days) was significant ($P < 0.005$) in comparison with the controls (median survival time, 56 days).

The main finding in the studies reported here was that BCG CW vaccine was at least as effective as living BCG in producing significant prolongation of survival of guinea pigs with a transplanted hepatoma at a time when there was local spread of disease to draining lymph nodes and artificial dissemination of tumor cells into the viscera. The availability of CW vaccine, which is easier to store and standardize and less

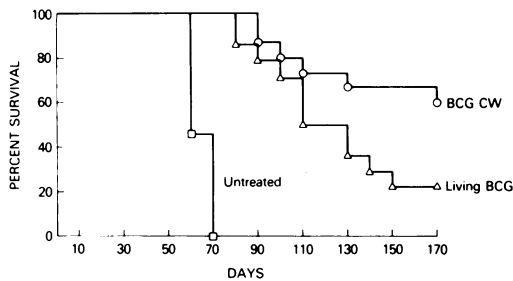


FIG. 1. Percent survival of guinea pigs with dermal tumors (10^6 tumor cells on day 0) and artificial hematogenous metastases (5×10^4 tumor cells on day 6) after intratumoral administration (day 7) of CW vaccine (○) or living BCG (△); untreated control (□). Nine of 15 animals treated with CW survived until the end of the experiment; 1 of the 9 had lymph node metastasis. The cure rates were evaluated statistically as follows: living BCG versus control, $P > 0.05$; CW versus control, $P < 0.01$; living BCG versus CW, $P > 0.05$. The statistical evaluation of prolongation of survival was as follows: living BCG versus CW, $P < 0.025$; living BCG versus control, $P < 0.001$; CW versus control, $P < 0.001$.

toxic than living BCG (B. Zbar, J. Hunter, H. J. Rapp, and G. Canti, *Cancer*, in press), may facilitate trials of cancer immunotherapy.

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